

ผลของสารสกัดใบบัวหลวง (*Nelumbo nucifera* Gaertn.)
ต่อสมรรถนะการเจริญเติบโตของปลานิล (*Oreochromis niloticus*)**
Effect of *Nelumbo nucifera* Gaertn. Leaf Extract on Growth Performance of
Nile Tilapia (*Oreochromis niloticus*)**

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บทคัดย่อ

บัวหลวง (*Nelumbo nucifera* Gaertn., Family Nelumbonaceae) ถูกนำมาใช้เพื่อการรักษาอาการเจ็บป่วยต่างๆ มาอย่างยาวนาน อย่างไรก็ตามการใช้บัวหลวงเพื่อเป็นวัตถุดิบธรรมชาติที่เสริมในอาหารปลายังคงมีน้อย ดังนั้น วัตถุประสงค์ของงานวิจัยนี้เพื่อศึกษาผลของสารสกัดใบบัวหลวงต่อสมรรถนะการเจริญเติบโตและผลข้างเคียงที่อาจเกิดขึ้นในปลานิล (*Oreochromis niloticus*) ทำการสกัดสารจากใบบัวหลวงด้วยแอลกอฮอล์ ปลานิลได้รับอาหารผสมสารสกัดใบบัวหลวงที่ระดับความเข้มข้นร้อยละ 0, 0.05, 0.1 และ 1 เปรียบเทียบกับปลานิลที่ได้รับอาหารผสมยาปฏิชีวนะออกซิเตตราซัยคลินที่ระดับความเข้มข้นร้อยละ 0.05 เป็นเวลา 8 สัปดาห์ ผลการศึกษาพบว่า การเสริมสารสกัดใบบัวหลวงลงในอาหารมีผลเพิ่มอัตราการเจริญเติบโตและการกินของปลานิลได้ รวมทั้งมีผลลดอัตราการเปลี่ยนอาหารเป็นเนื้ออย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) นอกจากนี้ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติของอัตราการตายสะสมและดัชนีน้ำหนักตัวต่อน้ำหนักตัวในปลาที่ได้รับสารสกัดเมื่อเปรียบเทียบกับกลุ่มควบคุมและกลุ่มที่ได้รับอาหารผสมออกซิเตตราซัยคลิน ระดับความเข้มข้นที่เหมาะสมของสารสกัดใบบัวหลวงจากการศึกษาในครั้งนี้ คือ ร้อยละ 1 ทั้งนี้ไม่พบความแตกต่างของพฤติกรรมการกินอาหาร ลักษณะที่สังเกตได้จากภายนอก และสุขภาพของปลาในแต่ละกลุ่มการทดลอง จึงชี้ให้เห็นว่าสารสกัดใบบัวหลวงมีความปลอดภัย ดังนั้น ผลการศึกษานี้จึงสนับสนุนการใช้สารสกัดใบบัวหลวงในการเป็นวัตถุดิบธรรมชาติที่เสริมในอาหารเพื่อกระตุ้นการเจริญเติบโตของปลา

คำสำคัญ : บัวหลวง สมรรถนะการเจริญเติบโต ปลานิล สมุนไพร

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Effect of *Nelumbo nucifera* Gaertn. Leaf Extract on Growth Performance of
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Abstract

Nelumbo nucifera Gaertn. (Family Nelumbonaceae) has been used for the treatment of various ailments. However, its use in fish culture as a natural feed additive to enhance fish growth is scant. Therefore, the aims of this present research were to examine the effects of *N. nucifera* leaf etharotic extract (NNLE) on growth performance and its adverse effects on Nile tilapia (*Oreochromis niloticus*). During an eighth week trial period, fish were fed diets containing NNLE at the levels of 0, 0.05, 0.1 and 1% compared with the diet containing oxytetracycline 0.05%. The results showed that the NNLE supplementation produced a significant increase in growth rate and feed intake and caused a significant decrease in feed conversion ratio ($p \leq 0.05$). There were no significant changes in the cumulative mortality and the hepatosomatic index of the experimental groups when compared to the control and oxytetracycline groups. The optimal concentration of NNLE observed in this research was 1%. Feeding behavior, external appearance and health of fish did not differ between the groups, indicating that NNLE is safe. Thus, these findings support the use of NNLE as a natural feed additive to improve growth performance of fish.

Keywords : *Nelumbo nucifera* Gaertn., Growth Performance, *Oreochromis niloticus*, Medicinal Plants

Introduction

Aquaculture industry is rapidly growing and it serves as an important protein source for human consumption worldwide. Intensive aquaculture production in the modern farming systems has given rise to the need to promote fish health and production. The use of antibiotics and growth promoting agents in aquaculture has many benefits related to animal health and well-being as well as to the economics of aquaculture producers (Citarasu, 2010; Harikrishnan et al., 2011). However, drugs and their residues could impose the potential risk on the consumers and cause antibiotic-resistant microorganisms in animals (Citarasu, 2010; Harikrishnan et al., 2011). Thus, alternatives to drug use in food animal production are required (Munglue, 2014).

Many studies have shown that the application of natural products as feed additives could increase growth performance and feed utilization of aquatic animals (Citarasu, 2010; Harikrishnan et al., 2011; Manoruang, 2014; Munglue, 2014; Phommanivong & Doolchidachabaporn, 2013). The beneficial effects of medicinal plants and their compounds on fish growth have been developed with varying amounts of success (Citarasu, 2010; Harikrishnan et al., 2011; Munglue, 2014). Munglue (2014) revealed that *N. nucifera* Gaertn. peduncle extract can improve the growth and feed utilization of Nile tilapia. Mahdavi et al. (2013) reported that common carp fed with *Aloe barbadensis* Miller extract mixed feed significantly enhanced growth performance and final weight in fish. It has been found that tilapia fed with a diet supplemented with quercetin, a plant flavonoid, at different levels caused a raised final weight gain and specific growth rate relative to the control group (Zhai & Liu, 2013). Fish fed diets containing a triterpenoid saponin of *Quillaja saponaria* showed a significant increase in average body weight, specific growth rate and utilization efficiency indices when compared to the control (Francis, Makkar & Becker, 2002; Francis, Makkar & Becker, 2005; Serrano, 2013).

N. nucifera Gaertn. (Family Nelumbonaceae) is a perennial aquatic plant. The different parts of *N. nucifera* Gaertn. have long been used for various therapeutic purposes worldwide (Mukherjee et al., 2009; Mukherjee et al., 2010; Sivagurunathan et al., 2012; Sridhar & Bhat, 2007). The main classes of phytochemical compounds found in *N. nucifera* Gaertn. are alkaloids, flavonoids, glycosides, triterpenoids and polyphenols (Ahn et al., 2013; Mukherjee et al., 2009; Mukherjee et al., 2010; Sivagurunathan et al., 2012; Sridhar & Bhat, 2007). In traditional medical systems, seeds of *N. nucifera* Gaertn. are used for hyperdipsia, fever, dermatopathy and antifertility (Mukherjee et al., 2009; Mukherjee et al., 2010; Munglue, 2014; Sivagurunathan et al., 2012; Sridhar & Bhat, 2007; Wethangkaboworn & Munglue, 2014). Leaves are useful for alleviating hematemesis, hemoptysis, hyperlipidemia, diabetic, and fever (Sridhar & Bhat, 2007). Seeds and leaves of *N. nucifera* Gaertn. have been reported to have antioxidant, antiinflammatory, antimicrobial activities and immunomodulatory (Kim & Shin, 2012; Mukherjee et al., 1997; Mukherjee et al., 2009; Mukherjee et al., 2010; Sridhar & Bhat, 2007). However, there is little information available regarding the effect of *N. nucifera* Gaertn. as a natural feed additive in food animal production to enhance the growth performance of fish (Munglue, 2014). Sivagurunathan et al. (2012) investigated the immunomodulatory effect of the dry powder extract of *N.*

nucifera Gaertn. mixed with diets on growth and hematology of *Cirrhinus mrigala* exposed to *Pseudomonas aeruginosa*. They found that *N. nucifera* Gaertn. extract significantly increased growth performance, feed utilization and haematological parameters such as total erythrocyte count, haemoglobin, total leucocyte count, lymphocyte and monocyte counts compared to the control diet (Sivagurunathan et al., 2012).

Thailand is one of the world's largest fish producers and exporters. In the last decade, Thailand has produced around 300,000 tons of Nile tilapia annually (Bhujel & Woollard, 2011). Nile tilapia culture in this country is widely distributed in rural areas and provides as an affordable protein source that may be consumed by local families and sold in local markets. Interestingly, Nile tilapia farming is now faced with the problem of rising feed prices and several opportunistic fish diseases (Bhujel & Woollard, 2011). Thus, more research still needs to be done on the effective methods for improving fish growth and feed utilization efficiency.

Aims

1. To investigate the effects of NNLE on growth performance of Nile tilapia
2. To investigate the adverse effects of NNLE on Nile tilapia

Materials and Methods

1. Plant Preparation

Fresh leaves of *N. nucifera* Gaertn. were collected locally from gardens in Sirindhorn district, Ubon Ratchathani, Thailand. The plant was identified by a herbal specialist at the Program of Biology, Ubon Ratchathani Rajabhat University, Thailand.

2. Plant Extraction

The samples of fresh *N. nucifera* Gaertn. leaves were cleaned using tap water, cut into small pieces and dried in a hot air oven at 45°C for five days. Dried leaf samples (50 g) were macerated with ethanol 70% (500 mL) for seven days. The extract was filtered through Whatman paper No. 1 and dried by rotary evaporator. The yield was 5.88 mg/g based on dried leaf weight.

3. Experimental Fish Preparation

The animal procedures were conducted in accordance with the Institutional Animal Care and Use Committee, Ubon Ratchathani Rajabhat University, Thailand. Fingerlings of Nile tilapia (weighting 9.76 ± 0.21 g) were obtained from Ubon Ratchathani Fishery Cooperatives, Ubon Ratchathani and acclimated in the Laboratory for two weeks before the experiment. Fish were randomly divided into 20 fish per 200 L aquarium after recording the individual wet weight. Each aquarium was aerated using aquarium air pumps. Fish waste was removed by siphoning one half of aquarium's water and replaced by aerated tap water from a storage tank. Water quality conditions were maintained in the standard ranges for Nile tilapia (data not shown). The fish diet was obtained from the commercial tilapia feed and contained no less than 25% protein and 3% lipid and was mixed with different doses of NNLE (0, 0.05, 0.1 and 1%) and oxytetracycline (0.05%). Fish were fed ad libitum two times a day for eight weeks. Every week, fish in each aquarium were weighed. Dead fish in aquaria were noted and removed (Munglue, 2014).

4. Effects on Growth Performance

At the end of the eight week experiment, the fish were fasted for 24 h before obtaining data. Growth parameters were calculated using the following formula (Munglue, 2014; Phommanivong & Doolchidachabaporn, 2013):

Weight gain (WG, %) = $100 \times (\text{final fish weight (g)} - \text{initial fish weight (g)}) / \text{initial fish weight (g)}$

Specific growth rate (SGR, %) = $100 \times [\ln \text{ final wet weight (g)} - \ln \text{ initial wet weight (g)}] / \text{experimental days}$.

Feed conversion ratio (FCR) = $\text{feed intake (g)} / \text{weight gain (g)}$.

Feed conversion efficiency (FCE) = $\text{weight gain (g)} / \text{feed intake (g)}$.

Survival rate (SR, %) = $100 \times (\text{final number of fish} / \text{initial number of fish})$.

Hepatosomatic index (HSI, %) = $100 \times (\text{weight of liver (g)} / \text{weight of fish (g)})$

5. Adverse Effects of Plant Extract

Fish in each group were observed daily for any sign of toxicity. The external appearance of the fish body and feeding behavior were also noted (Munglue, 2014).

6. Statistical Analysis

Data are expressed as mean \pm standard error of the mean (SEM). The significance of difference was analyzed using one-way analysis of variance (ANOVA). p value ≤ 0.05 was considered statistically significant.

Results and Discussion

1. Effects on Growth Performance

The effects of NNLE on the performance of fish and the feed utilization are presented in Table 1. The SR of all Nile tilapia fed with the experimental diets were determined at the end of the eight weeks. There were no significant differences of the SR between the treatment and the control groups. Dietary supplementations with NNLE and oxytetracycline produced a significant increase in final weight, WG, SGR and FCE ($p \leq 0.05$). In addition, FCR values were significantly decreased in the experimental diets when compared to the control diet ($p \leq 0.05$). The different concentrations of NNLE did not significantly alter the HSI values. The results also indicated that the optimal concentration of NNLE observed in this experiment was 1%.

That medicinal plants can act as growth promoter could be attributed to some classes of active compounds such as alkaloids, flavonoids, triterpenoids and saponins (Citarasu, 2010; Harikrishnan et al., 2011). A recent report indicated that dietary supplementation of triterpene saponins of *Quillaja* produced a significant increase in the final weight, WG, FCR, average protein utilization, and metabolic growth rate of common carp compared to the control group (Francis et al., 2002). It has been hypothesized that dietary saponins may affect some specific enzymes in the gastrointestinal tract such as trypsin, amylase, cytochrome c-oxidase and lactate dehydrogenase to enhance the permeability of the gut membrane, leading to an increased efficiency of digestion and absorption of essential nutrients (Francis et al., 2002; Francis et al., 2005; Serrano, 2013). In addition, Zhai & Liu (2013) reported that a fish diet supplemented with quercetin compound produced a significant increase in SGR and condition factor and caused a significant decrease in FCR in Nile tilapia when compared to the control diet without the quercetin compound. It is suggested that the growth-promoting influence of quercetin supplementation may be due to the activation of specific digestive enzymes and immune cells in animals, resulting in the optimum growth, health and feed utilization (Zhai & Liu,

Table 1 Growth performance and survival rate of Nile tilapia fed the diets containing NNLE at the different levels for 8 weeks.

Parameter	Treatment				
	Control	0.05% NNLE	0.1% NNLE	1% NNLE	0.05% Oxy
Initial weight (g)	9.78±0.81 ^a	9.75±0.72 ^a	9.72±0.67 ^a	9.76±0.78 ^a	9.80±0.70 ^a
Final weight (g)	13.02±0.02 ^a	15.30±0.17 ^{ab}	16.03±0.20 ^b	19.05±0.12 ^c	16.45±1.30 ^b
WG (%)	43.04±4.56 ^a	55.85±6.25 ^{ab}	64.91±3.40 ^b	74.69±3.32 ^c	67.85±5.64 ^b
SGR (%)	0.51±0.07 ^a	0.79±0.05 ^b	0.89±0.05 ^b	0.98±0.04 ^c	0.92±0.03 ^b
FCR	1.75±0.04 ^a	1.60±0.03 ^b	1.52±0.04 ^c	1.45±0.05 ^d	1.50±0.06 ^b
FCE	2.55±0.02 ^a	2.62±0.02 ^b	2.67±0.01 ^{bc}	2.72±0.03 ^c	2.66±0.04 ^{bc}
HSI (%)	2.98±0.81 ^a	3.07±0.89 ^a	3.05±0.96 ^a	3.03±0.95 ^a	3.06±0.82 ^a
SR (%)	97.01±0.32 ^a	95.06±0.51 ^a	95.07±0.85 ^a	96.04±0.37 ^a	95.23±1.20 ^a

NNLE = *N. nucifera* Gaertn. leaf extract, Oxy = Oxytetracycline, WG = Weight gain, SGR = Specific growth rate, FCR = Feed conversion ratio, FEC = Feed conversion efficiency, HSI = Hepatosomatic index, SR = Survival rate, Values are expressed as mean ± SEM. *n* = 8 (number of animals in each group). One-way analysis of variance (ANOVA) was used. Means with different superscripts ^(a-d) in the same row are significantly different ($p \leq 0.05$).

2013). There is evidence that Nile tilapia, common carp and African catfish (*Clarias gariepinus*) fed diets containing various levels of red clover (*Trifolium pretense*) extract had significantly enhanced final weight, WG, SGR and protein and lipid contents (Turan, 2006; Turan & Akyurt, 2005; Turan, Gurlek & Yaglioglu, 2007). It was suggested that phytochemicals such as oestrogenic isoflavones found in red clover can increase the levels of growth hormone and insulin-like growth factor in fishing lambs, leading to an increased growth rate in these animals (Moorby et al., 2004). Therefore, the growth promoting property of red clover observed in aquatic animals, would follow a mechanism of action similar to those postulated in mammalian models (Moorby et al., 2004; Turan, 2006; Turan & Akyurt, 2005; Turan et al., 2007).

Previous research found flavonoids, steroids, alkaloids, terpenoids and saponins to be the main compounds of *N. nucifera* Gaertn. leaves (Ahn et al., 2013; Mukherjee et al., 2009; Mukherjee et al., 2010; Sridhar & Bhat, 2007). As mentioned above, saponins and flavonoids increase growth of fish (Francis et al., 2002; Francis et al., 2005; Serrano, 2013; Zhai & Liu, 2013). Thus, growth-promoting effects of dietary NNLE on Nile tilapia could be attributed largely to some chemical compounds found in the plant extract including flavonoids and saponins. Based on several scientific reports, the growth promoting effects of NNLE on Nile tilapia might be due to: 1) the improvement of palatability (Francis et al., 2002; Francis et al., 2005; Sivagurunathan et al., 2012), 2) the increased activities of gastrointestinal enzymes to enhance digestion and absorption of nutrients (Francis et al., 2002; Francis et al., 2005; Serrano, 2013; Zhai & Liu, 2013), 3) the improvement of gastrointestinal microflora of fish (Dibner & Richards, 2005), 4) the increased modulation of the immune functions in order to protect fish from various pathogens and diseases (Sivagurunathan et al., 2012), and 5) antimicrobial, antioxidative and anti-inflammatory activities of the plant extract which may be associated with the improvement of overall fish health and performance (Kim & Shin, 2012; Mukherjee et al., 1997; Sittiwet, 2009).

In the present study, fish fed with the dietary supplement NNLE displayed significant increases in growth parameters such as final weight, WG and SGR. The optimal NNLE concentration observed was determined to be 1%. The plant extract did not significantly affect the SR and the HSI compared with the control group. These results are consistent with the earlier study of Sivagurunathan et al. (2012) who reported that *N. nucifera* Gaertn. extract produced a significant increase in the SGR and FCR in *Cirrhinus*

mrigala. In addition, Munglue (2014) demonstrated that the *N. nucifera* Gaertn. peduncle extract can improve the growth and feed utilization of Nile tilapia, indicating that *N. nucifera* Gaertn. can be used as a natural growth promoter in the fish production industry.

2. Adverse Effects of Plant Extract

The results demonstrate that the plant extract did not produce any side-effects during the experimental period. In addition, treated fish did not change their general behavior. The external characteristics and feeding behavior of fish fed with the diets containing the herbal plant extract were similar to those of the control fish, which suggested that NNLE can be used for a longer time without producing treatment-related adverse effects (Munglue, 2014).

Conclusion

The results of this study indicate that dietary NNLE can improve growth performance of Nile tilapia. During the experimental period, no adverse effects on external appearance of the fish body and feeding behavior were observed. The suitable concentration of NNLE observed was 1%. The bioactive compounds related to the growth promoting effects of the plant extract may be flavonoids and saponins. This study suggests that NNLE can be used as a natural growth promoting agent to enhance growth performance of cultured fish.

Suggestion

Further research is needed for the identification and isolation of the active compounds of *N. nucifera* Gaertn. responsible for improving the growth of fish and mode of actions.

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